REMARKS

Reconsideration of the present application in view of the above amendments and the following remarks is respectfully requested.

Claims 1, 2, 6-9 and 14-34 are pending, of which claims 1, 2, 14-27, 33 and 34 are under consideration. Claims 1, 9, 20, 23 and 25-27 have been amended to clarify the claimed subject matter.

The specification has been amended to correct typographical errors. Support for the amendments may be found throughout the present application, for example, in the second paragraph at page 5, SEQ ID NOS:2 and 4, and the third full paragraph on page 11.

Election/Restrictions

Applicants respectfully request that the Restriction Requirement be reconsidered and withdrawn.

The Restriction Requirement appears to be based on the following interpretation of the pending claims: "applicants recitation of a mutation position at Q879, is interpreted as a limitation of the referred to Klenow fragment thereof to which the mutated activity is compared not a limitation of the claimed mutation itself" (*see*, the second paragraph on page 2 of the Office Action). Applicants submit that this above-noted interpretation is incorrect. SEQ ID NO:2, which is the amino acid sequence of *E. coli* Klenow fragment of DNA polymerase 1 (*see*, the table regarding the sequence listing on page 8 of the present application), has QVH at positions 879-881. In addition, Figure 1 of the present application and Minnick *et al.* (Journal of Biological Chemistry 274(5): 3067-3075, 1999, "Minnick") also show that Q879 is the wild type. Accordingly, one of ordinary skill in the art, in view of the present application, would interpret that the recited mutation of Q879 is a limitation of the claimed mutation itself, not a limitation of a wild type Klenow fragment. Nevertheless, to facilitate allowance, Applicants have amended claim 1 to clearly indicate that Q879 is the amino acid at position 879 in a wild type motif C sequence, and that the substitution of Q879 by a lipophilic amino acid is in the modified motif C sequence.

Applicants further submit that the family A DNA polymerase according to amended claim 1 is novel and not obvious and thus may function as the specific technical feature that links the three groups of invention. More specifically, Minnick does not teach or suggest a family A DNA polymerase which has a modified motif C sequence and an enhanced mismatch discrimination as compared to the corresponding wild type polymerase, or a Klenow fragment thereof, wherein in the modified motif C sequence, at least the amino acid residue Q879 in the wild type motif C sequence QVH in positions 879-881 of the *E. coli* DNA polymerase Klenow fragment shown in SEQ ID NO:2 has been replaced by a lipophilic amino acid residue. Although this reference discloses a His to Ala substitution at position 881, it fails to disclose any substitution of the amino acid residue Q879. In addition, the present application provides that "[a]s compared to the mutant QVA known from the literature (Minnick, T. et al., J. Biol. Chem. 274, 3067-3075 (1999)), the polymerase mutants according to the invention have an increased selectivity of primer extension (Example 7, Figure 5). As shown in Figure 5, the QVA mutant has an higher tendency to extend mismatches as compared to the LVL mutant according to the invention. Thus, the family A DNA polymerase claimed in the present application is unexpectedly superior to and not obvious in view of the DNA polymerase described in Minnick.

Information Disclosure Statement

It is stated in the Office Action that "[t]he listing of references in the specification is not a proper information disclosure statement."

Applicants are unclear about to which listing of references in the specification is objected and respectfully request that the Examiner clarify this objection.

Rejection Under 35 U.S.C. 112 (Indefiniteness)

Claims 1, 2, 14-27, 33 and 34 stand rejected under 35 U.S.C. 112, second paragraph, as indefinite. More specifically, it is asserted in the Office Action that the recitation " . . . at least the amino acid residue Q879 has been replaced by a lipophilic amino acid residue in claim 1" is confusing because it is unclear if this recitation describes the claimed family A DNA polymerase mutant or the corresponding wild type polymerase or a Klenow fragment thereof.

Applicants respectfully traverse this ground of rejection. As discussed above, one of ordinary skill in the art would interpret the above-noted language as describing the claimed family A DNA polymerase mutant or its Klenow fragment, not the corresponding wild type polymerase or its Klenow fragment. Nevertheless, to facilitate allowance, Applicants have amended claim 1 to further clarify the claimed family A DNA polymerase or its Klenow fragment.

In view of the above remarks, Applicants respectfully submit that this ground of rejection under 35 U.S.C. 112, second paragraph, has been overcome and request that this rejection be withdrawn.

Rejection Under 35 U.S.C. 112 (Written Description)

Claims 1, 2, 14-27, 33 and 34 stand rejected under 35 U.S.C. 112, first paragraph, as failing to meet the written description requirement. More specifically, it is stated in the Office Action that

> The specification, however, only provides the representative species of Taq and E. coli DNA polymerase mutants encompassed by these claims. There is no disclosure of any particular structure to function/activity relationship in the single disclosed species. The specification also fails to describe additional representative species of these family A DNA polymerases by any identifying structural characteristics or properties other than the activities in claims 1, for which no predictability of structure is apparent (See also above rejection under 112 second paragraph). While applicants have functionally defined the claims in terms of the result of a motif C modification of a family A DNA polymerase, the claims are not limited structurally in any way, such that since the claims are drawn to a family A DNA polymerase which has been modified, the claims no longer have any structural limitations. Given this lack of species representative of such an unlimited genus of modified DNA polymerases, as encompassed by the claims, Applicants have failed to sufficiently describe the claimed invention, in such full, clear concise, and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention.

Applicants respectfully traverse this ground of rejection. The written description requirement is met if the applicant conveys to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64; 19 USPQ 1111 (Fed. Cir. 1991). "A claim will not be invalidated on section 112 grounds simply because the embodiments of the specification do not contain examples

explicitly covering the full scope of the claim language. This is because the patent specification is written for a person of skill in the art, and such a person comes to the patent with the knowledge of what has come before. Placed in that context, it is unnecessary to spell out every detail of the invention in the specification; only enough must be included to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation." *Lizard-Tech, Inc. v. Earth Resource Mapping, PTY, Inc.*, 424 F.3d 1336, 1345; 76 USPQ2d 1724 (Fed. Cir. 2005).

Claim 1 of the present application is directed to a family A DNA polymerase or its Klenow fragment that has a modified motif C sequence and an enhanced mismatch discrimination as compared to the corresponding wild type polymerase or its Klenow fragment. In the modified motif C sequence, at least the amino acid residue Q879 in the wild type motif C sequence QVH in positions 879-881 of the *E. coli* DNA polymerase Klenow fragment shown in SEQ ID NO:2 has been replaced by a lipophilic amino acid residue. The term "family A DNA polymerase" is defined in the present application as referring to DNA polymerizing enzymes that contain the A motif with the sequence DYSQIELR in their active site. Thus, the family A DNA polymerase or its Klenow fragment according to claim 1 of the present application has the following two structural features: (1) it has the A motif with the sequence DYSQIELR in its active site, and (2) it has a modified motif C sequence in which at least the amino acid residue Q879 in the wild type motif C sequence QVH in positions 879-881 of the *E. coli* DNA polymerase Klenow fragment has been replaced by a lipophilic amino acid residue. In addition, the family A DNA polymerase or its Klenow fragment claimed in the present application has the following two functional features: (1) it has DNA polymerase activity, and (2) it has an enhanced mismatch discrimination as compared to the corresponding wild type polymerase or its Klenow fragment.

Applicants submit that one of ordinary skill in the art would not doubt that the present inventors had possession of the claimed invention at the filing of the present application. First, such a person would not doubt that the inventors had possession of a family A DNA polymerase. Applicants disagree with the assertion in the Office Action that the claims are not limited structurally in any way. As indicated above, because the present application defines the term "family A DNA polymerase" as referring to a DNA polymerase that contain the A motif

with the sequence DYSQIELR in its active site, the claimed family A DNA polymerase or its Klenow fragment has motif A with the sequence DYSQIELR in its active site. Applicants submit that family A DNA polymerases and its Klenow fragments were well known and characterized in the art at the filing of the present application. More specifically, as indicated in Patel *et al.* (J. Mol. Biol. 308:823-837, 2001, "Patel," copy enclosed), over 50 family A polymerases from different prokaryotic species had been sequenced (*see*, Abstract). Sequence alignments of these and other related polymerases show six conserved regions that form the active sites with regions 3, 4 and 5 corresponding to most conserved motifs A, B and C, respectively (*see*, pages 825 and 826, Figures 2 and 3). In addition, high-resolution crystal structures of several DNA polymerases had also been determined (*see*, page 824 and Figures 1 and 4-7). These studies in combination with mutagenesis analyses illustrated the structure-function relationship of family A DNA polymerases, including the function of individual amino acid residues in polymerization (*see*, pages 832-834). Thus, although not explicitly described in the present application, numerous family A DNA polymerases and their structure-function relationship were known in the art. In view of the advance understanding of family A DNA polymerases in the art at the time the present application was filed, one of ordinary skill in the art would not doubt that the present inventors had possession of a family A DNA polymerase other than Taq and *E. coli* DNA polymerases explicitly described in the present application.

Second, one of ordinary skill in the art would not doubt that the present inventors had possession of a family A DNA polymerase with an enhanced mismatch discrimination as compared to the corresponding wild type polymerase. As discussed above, the claimed DNA polymerase or its Klenow fragment has an additional structural feature: It has a modified motif C sequence in which at least the amino acid residue Q879 in the wild type motif C sequence QVH in positions 879-881 of the *E. coli* DNA polymerase Klenow fragment has been replaced by a lipophilic amino acid residue. The present application provides numerous exemplary substitutions of the QVH sequence (*see*, the third full paragraph on page 11). It further shows that certain exemplary substitutions of the QVH sequence resulted in enhanced mismatch discrimination activity of the modified DNA polymerases (*see*, Examples 3, 5 and 6, Figures 1-4). In view of such disclosure provided by the present application, Applicants submit that one of ordinary skill in the art would not doubt the possession of a family A DNA polymerase with an

enhanced mismatch discrimination as compared to the corresponding wild type polymerase by the present inventors.

In view of the above remarks, Applicants submit that this ground of rejection under 35 U.S.C. 112, first paragraph, has been overcome. Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. 112 (Enablement)

Claims 1, 2, 14-27, 33 and 34 stand rejected under 35 U.S.C. 112, first paragraph, as not enabled. More specifically, it is asserted in the Office Action that the specification, while enabling for a family A DNA polymerase comprising the amino acid sequence of SEQ ID NO:2 in which the amino acid residue at position Q879 has been replaced with a lipophilic amino acid residue, does not reasonably provide enablement for any family A DNA polymerases that have a modified motif C sequence and an enhanced mismatch discrimination as compared to a corresponding wild type polymerase or its Klenow fragment in which at least the amino acid residue Q879 in the motif C sequence QVH at positions 879-881 based on the *E. coli* DNA polymerase Klenow fragment has been replaced by a lipophilic amino acid residue.

Applicants respectfully traverse this ground of rejection. It seems that the non-enablement rejection in the Office Action is partially based on the assertion that the present claims place minimal if any structural limits on the claimed modified polymerases. As discussed above, Applicants respectfully disagree with this assertion. The claimed DNA polymerase has the following two structural features: (1) it has the A motif with the sequence DYSQIELR in its active site, and (2) it has a modified motif C sequence in which at least the amino acid residue Q879 in the wild type motif C sequence QVH in positions 879-881 of the *E. coli* DNA polymerase Klenow fragment has been replaced by a lipophilic amino acid residue. The first structural feature relates to the DNA polymerase activity of the claimed modified family A DNA polymerase, and the second structural feature relates to the enhanced mismatch discrimination activity of the claimed modified family A DNA polymerase. As discussed in detail below, one of ordinary skill in the art would know how to make (and use) the claimed DNA polymerase in view of the present application in combination of knowledge in the art available when the present application was filed.

First, one of ordinary skill in the art would know how to make (and use) a family A DNA polymerase based on the knowledge available at the filing of the present application. As discussed above, family A DNA polymerases were well known, and their structure-function relationship was well characterized. For example, as described in Patel, family A DNA polymerases have 6 conservative regions that form their active sites, and the tertiary structures of several family A DNA polymerases were characterized. In addition, function of individual amino acids was further studied, which shows that very few (<10) amino acid residues within the highly conserved motifs A, B and C have a direct role during nucleotide binding and incorporation, and only those residues are important during catalysis and/or for protein folding need to be maintained, while all other residues are mutable (*see*, the Conclusions section on page 835, the first full paragraph in the right column on page 833 and the first paragraph in the right column on page 834, and the second paragraph in the left column on page 835). Thus, both the regions of family A DNA polymerases that may be modified without effecting polymerase activities and the great tolerance of this type of polymerase to modification were known in the art. In view of such knowledge in the art, one of ordinary skill in the art would be able to make family A DNA polymerases, including both naturally occurring family A DNA polymerases and their variants with retained DNA polymerase activity.

Applicants submit that the reference cited in the Office Action, Ngo, is insufficient to support the non-enablement rejection. First, this reference was published in 1994, 10 years earlier than the priority date of the present application. Thus, it does not describe the state of art at the time of the present application. Second, Ngo states that it is not known whether there exists an efficient algorithm for predicting the structure of a given protein form its amino acid sequence alone (*see*, second full paragraph on page 492). However, computer prediction via algorithms is not the only way that the structure of a given protein may be analyzed. As indicated in Patel, high-resolution crystal structures of several polykaryotic DNA polymerases have been determined, and the structure and function relationship of these enzymes have been characterized by various means, including sequence alignments, crystal structural analysis, and mutagenesis studies. Thus, Ngo does not specifically describe the state of the art regarding polykaryotic DNA polymerases.

Second, one of ordinary skill in the art would also know how to modify a family A DNA polymerase to increase its mismatch discrimination activity in view of the present application. The present application provides that mismatch discrimination activity of a family A DNA polymerase may be enhanced by replacing Q879 in the motif C sequence QVH with a lipophilic amino acid residue, such as Gly, Ala, Val, Leu and Ile (*see, e.g.*, second paragraph on page 5 and third full paragraph on page 11). Example 1 of the present application teaches how to make and purify Klenow fragment variants with substitution in the motif C sequence QVH. Example 2 of the present application teaches how to screen the Klenow fragment variants for those with an enhanced mismatch discrimination activity. Examples 3, 5 and 6 demonstrate that exemplary variants containing substitutions in the motif C sequence QVH have enhanced mismatch discrimination activity in primer extension assays and real time PCR experiments. In view of the teachings of the present application, one skilled in the art would be able to make a family A DNA polymerase variant having Q879 replaced with a lipophilic amino acid residue (*see*, Example 1) and determine its mismatch discrimination activity (*see*, Examples 2, 3, 5 and 6) without undue experimentation.

In view of the above remarks, Applicants submit that this ground of rejection under 35 U.S.C. 112, first paragraph, has been overcome. Withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. 102(b) (Novelty)

Claims 1, 2, 14-27, 33 and 34 stand rejected under 35 U.S.C. 102 as anticipated by Minnick.

Applicants respectfully traverse this ground of rejection. As discussed above, the Minnick does not teach or suggest a family A DNA polymerase which has a modified motif C sequence in view at least the amino acid residue Q879 in the wild type motif C sequence QVH in positions 879-881 of the *E. coli* DNA polymerase Klenow fragment shown in SEQ ID NO:2 has been replaced by a lipophilic amino acid residue. Although this reference discloses a His to Ala substitution at position 881, it fails to disclose any substitution of the amino acid residue Q879.

In view of the above remarks, Applicants submit that this ground of rejection has been overcome. Withdrawal of this rejection is respectfully requested.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

/Qing Lin/
Qing Lin, Ph.D.
Registration No. 53,937

Enclosure:
Patel *et al.*, J. Mol. Biol. 308:823-837, 2001

QXL:tl

701 Fifth Avenue, Suite 5400
Seattle, Washington 98104
Phone: (206) 622-4900
Fax: (206) 682-6031

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